

Amendments to the Claims

This listing of claims replaces all prior versions, and listings, of claims in the above-identified application:

1. (Currently Amended) An isolated polynucleotide encoding a replication competent hepatitis C virus genotype 1a replicon comprising:
a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, wherein the polyprotein comprises an amino acid sequence having at least about 95% identity to amino acids 1 – 3011 of SEQ ID NO:2, and wherein the amino acid sequence of the polyprotein comprises at least three adaptive mutations, wherein the adaptive mutations comprise an isoleucine at about amino acid 2204 of the polyprotein and at least two adaptive mutations selected from the group of an arginine at about amino acid 1067 of the polyprotein, an arginine at about amino acid 1691 of the polyprotein, a valine at about amino acid 2080 of the polyprotein, an isoleucine at about amino acid 1655 of the polyprotein, an arginine at about amino acid 2040 of the polyprotein, an arginine at about amino acid 1188 of the polyprotein, and a combination thereof.
2. (Previously Presented) The isolated polynucleotide of claim 1 further comprising a second coding sequence.
3. (Previously Presented) The isolated polynucleotide of claim 2 wherein the second coding sequence encodes a marker.
4. (Previously Presented) The isolated polynucleotide of claim 2 wherein the second coding sequence encodes a transactivator.
5. Canceled

6. (Previously Presented) The isolated polynucleotide of claim 1 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.
7. (Previously Presented) The isolated polynucleotide of claim 1 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.
8. (Previously Presented) The isolated polynucleotide of claim 1 further comprising a nucleotide sequence having cis-acting ribozyme activity, wherein the nucleotide sequence is located 3' of the 3' NTR.
- 9-13. (Cancelled)
14. (Currently Amended) A method for making an isolated polynucleotide encoding a replication competent hepatitis C virus genotype 1a replicon comprising:
- providing a polynucleotide comprising a 5' NTR, 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, wherein the polyprotein comprises an amino acid sequence having at least about 95% identity to amino acids 1 – 3011 of SEQ ID NO:2, and wherein the amino acid sequence of the polyprotein comprises a serine at about amino acid 2204 of the polyprotein, a glutamine at about amino acid 1067 of the polyprotein, a lysine at about amino acid 1691 of the polyprotein, a phenylalanine at about amino acid 2080 of the polyprotein, a valine at about amino acid 1655 of the polyprotein, a lysine at about amino acid 2040 of the polyprotein, or a glycine at about amino acid 1188 of the polyprotein; and
- altering the coding sequence such that the polyprotein encoded thereby comprises at least three adaptive mutations, wherein the adaptive mutations comprise an isoleucine at about amino acid 2204 of the polyprotein and at least two adaptive mutations selected from the group consisting of an arginine at about amino acid 1067 of the polyprotein, an arginine at about amino acid 1691 of the polyprotein, a valine at about amino acid 2080 of the polyprotein, an isoleucine

at about amino acid 1655 of the polyprotein, an arginine at about amino acid 2040 of the polyprotein, an arginine at about amino acid 1188 of the polyprotein, and a combination thereof.

15. (Original) The method of claim 14 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

16. (Original) The method of claim 14 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

17. (Previously Presented) A replication competent hepatitis C virus genotype 1a replicon produced by the method of claim 14.

18. (Currently Amended) A method for identifying a compound that inhibits replication of a replication competent hepatitis C virus genotype 1a replicon, the method comprising:

contacting a cell comprising a replication competent hepatitis C virus genotype 1a replicon with a compound, the replication competent hepatitis C virus genotype 1a replicon comprising a 5' NTR, 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, wherein the hepatitis C virus polyprotein comprises an amino acid sequence having at least about 95% identity to amino acids 1 – 3011 of SEQ ID NO:2, and wherein the amino acid sequence of the polyprotein comprises at least three adaptive mutations, wherein the adaptive mutations comprise an isoleucine at about amino acid 2204 of the polyprotein and at least two adaptive mutations selected from the group of an arginine at about amino acid 1067 of the polyprotein, an arginine at about amino acid 1691 of the polyprotein, a valine at about amino acid 2080 of the polyprotein, an isoleucine at about amino acid 1655 of the polyprotein, an arginine at about amino acid 2040 of the polyprotein, an arginine at about amino acid 1188 of the polyprotein, and a combination thereof;

incubating the cell under conditions wherein the replication competent hepatitis C virus genotype 1a replicon replicates in the absence of the compound; and

detecting the replication competent hepatitis C virus genotype 1a replicon, wherein a decrease of the replication competent hepatitis C virus genotype 1a replicon in the cell contacted with the compound compared to the replication competent hepatitis C virus genotype 1a replicon in a cell not contacted with the compound indicates the compound inhibits replication of the replication competent hepatitis C virus genotype 1a replicon.

19. (Previously Presented) The method of claim 18 wherein detecting the replication competent hepatitis C virus genotype 1a replicon comprises nucleic acid amplification.

20. (Previously Presented) The method of claim 18 wherein the replication competent hepatitis C virus genotype 1a replicon further comprises a second coding sequence encoding a marker, and wherein detecting the replication competent hepatitis C virus genotype 1a replicon comprises identifying the marker.

21. (Previously Presented) The method of claim 18 wherein the replication competent hepatitis C virus genotype 1a replicon further comprises a second coding sequence encoding a transactivator, wherein the cell comprises a polynucleotide comprising a transactivated coding sequence encoding a detectable marker and an operator sequence operably linked to the transactivated coding sequence, wherein the transactivator interacts with the operator sequence and alters expression of the transactivated coding sequence, and wherein detecting the replication competent hepatitis C virus genotype 1a replicon in the cell comprises detecting the detectable marker encoded by the transactivated coding sequence.

22. (Original) The method of claim 18 wherein the cell is a human hepatoma cell.

23. (Original) The method of claim 18 wherein the hepatitis C virus polypeptide is a subgenomic hepatitis C virus polypeptide.

24. (Original) The method of claim 18 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

25. Canceled

26. (Currently Amended) A method for selecting a replication competent hepatitis C virus genotype 1a replicon, the method comprising:

incubating a cell in the presence of a selecting agent, wherein:

the cell comprises a polynucleotide comprising a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, and a second coding sequence, wherein the polyprotein comprises an amino acid sequence having at least about 95% identity to amino acids 1 – 3011 of SEQ ID NO:2, and wherein the amino acid sequence of the polyprotein comprises at least three adaptive mutations, wherein the adaptive mutations comprise an isoleucine at about amino acid 2204 of the polyprotein and at least two adaptive mutations selected from the group of an arginine at about amino acid 1067 of the polyprotein, an arginine at about amino acid 1691 of the polyprotein, a valine at about amino acid 2080 of the polyprotein, an isoleucine at about amino acid 1655 of the polyprotein, an arginine at about amino acid 2040 of the polyprotein, an arginine at about amino acid 1188 of the polyprotein, and a combination thereof;

the second coding sequence encodes a selectable marker conferring resistance to the selecting agent; and

the selecting agent inhibits replication of a cell that does not express the selectable marker; and

detecting a cell that replicates in the presence of the selecting agent, wherein the presence of such a cell indicates the polynucleotide is replication competent.

27. (Original) The method of claim 26 wherein the selecting agent is an antibiotic.

28. (Original) The method of claim 26 wherein the cell is a human hepatoma cell.

29. (Previously Presented) The method of claim 26 wherein the cell is a first cell, the method further comprising:

obtaining a virus particle produced by the first cell;

exposing a second cell to the isolated virus particle and incubating the second cell in the presence of the selecting agent; and

detecting a second cell that replicates in the presence of the selecting agent, wherein the presence of such a cell indicates the replication competent hepatitis C virus genotype 1a replicon in the first cell produces an infectious virus particle.

30. (Original) The method of claim 26 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

31. (Original) The method of claim 26 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

32. Canceled

33. (Currently Amended) A method for detecting a replication competent hepatitis C virus genotype 1a replicon, the method comprising:

incubating a cell comprising a replication competent hepatitis C virus genotype 1a replicon, wherein:

the replication competent hepatitis C virus genotype 1a replicon comprises a 5' non-translated region (NTR), a 3' NTR, and a first coding sequence present between the 5' NTR and 3' NTR and encoding a hepatitis C virus polyprotein, and a second coding sequence encoding a transactivator, wherein the polyprotein comprises an amino acid sequence having at least about 95% identity to amino acids 1 – 3011 of SEQ ID NO:2, and wherein the amino acid sequence of the polyprotein comprises at least three adaptive mutations, wherein the adaptive mutations comprise an isoleucine at about amino acid 2204 of the polyprotein and at least two adaptive mutations selected from the group of an arginine at about amino acid 1067 of the

polyprotein, an arginine at about amino acid 1691 of the polyprotein, a valine at about amino acid 2080 of the polyprotein, an isoleucine at about amino acid 1655 of the polyprotein, an arginine at about amino acid 2040 of the polyprotein, an arginine at about amino acid 1188 of the polyprotein, and a combination thereof;

the cell comprises a transactivated coding region and an operator sequence operably linked to the transactivated coding region; and

the transactivated coding region encodes a detectable marker, wherein the transactivator alters transcription of the transactivated coding region; and

detecting the detectable marker, wherein the presence of the detectable marker indicates the cell comprises a replication competent hepatitis C virus genotype 1a replicon.

34. (Original) The method of claim 33 wherein the hepatitis C virus polyprotein is a subgenomic hepatitis C virus polyprotein.

35. (Original) The method of claim 33 wherein the hepatitis C virus polyprotein comprises cleavage products core, E1, E2, P7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B.

36. Canceled

37-40. (Cancelled)

41. (Previously Presented) A method for producing viral particles, comprising incubating a cell comprising the replication competent hepatitis C virus genotype 1a replicon of claim 1 under conditions allowing the polynucleotide to replicate.

42. (Original) The method of claim 41 further comprising isolating the viral particles.

43. (Original) A method for using the viral particles of claim 41 in an assay.

44-46. (Cancelled)

47. (Previously Presented) The replication competent polynucleotide of claim 1 wherein the replication competent hepatitis C virus genotype 1a replicon replicates in Huh-7 cells.

48. (Previously Presented) The method of claim 14 wherein the replication competent hepatitis C virus genotype 1a replicon replicates in Huh-7 cells.

49. (Previously Presented) The method of claim 22 wherein the cell is a Huh-7 cell.

50. (Previously Presented) The method of claim 28 wherein the cell is a Huh-7 cell.

51. (Previously Presented) The method of claim 33 wherein the cell is a human hepatoma cell.

52. (Previously Presented) The method of claim 51 wherein the cell is a Huh-7 cell.

53. (Previously Presented) The isolated polynucleotide of claim 1 wherein at least three adaptive mutations are an isoleucine at amino acid 2204 of the polyprotein, an arginine at amino acid 1067 of the polyprotein, an arginine at amino acid 1691 of the polyprotein, a valine at amino acid 2080 of the polyprotein, an isoleucine at amino acid 1655 of the polyprotein, an arginine at amino acid 2040 of the polyprotein, and an arginine at amino acid 1188 of the polyprotein.